FDA U.S. FOOD & DRUG ADMINISTRATION

Our Reference: PS010700
Meeting ID #22239

MEETING SUMMARY
Date: March 27, 2026

Children's Hospital of Philadelphia (CHOP)
Attention: Rebecca Ahrens-Nicklas, MD, PhD
3501 Civic Center Blvd.
Philadelphia, PA 19104

Dear Dr. Ahrens-Nicklas:

Attached is a copy of the memorandum summarizing your February 27, 2026, Type B, pre-IND meeting with CBER. This memorandum constitutes the official record of the meeting. If your understanding of the meeting outcomes differ from those expressed in this summary, it is your responsibility to communicate with CBER as soon as possible.

Please include a reference to Meeting ID #22239 and PS010700 in your future submissions related to the subject product.

If you have any questions, please contact [redacted] [redacted].

Sincerely,

[redacted]

[redacted]
Division of Review Management and Regulatory Review 2
Office of Review Management and Regulatory Review
Office of Therapeutic Products
Center for Biologics Evaluation and Research

Meeting Summary
(Includes Preliminary Meeting Responses)

Meeting ID #:	22239
Submission type & #:	PS010700
Product name:	Lipid nanoparticles (LNPs) encapsulating an mRNA encoding a prime editor 6c (PE6c), and adeno-associated viral (AAV) vector encoding prime editing guide RNAs (pegRNAs) and nicking guide RNAs (ngRNAs) targeting the site(s) of either one or two pathogenic variants in the *NAGS*, *CPS1*, *OTC*, *ASS1*, *ASL*, *ARG*, or *SLC25A15* gene. Both are delivered to the hepatocytes in the liver as an IV infusion; Product Name: CHOP.UCD.PE
Proposed indication:	Treatment of hyperammonemia in patients [redacted]
Sponsor:	Children's Hospital of Philadelphia (CHOP)
Meeting type:	B
Meeting category:	Pre-IND
Meeting date & time:	February 27, 2026, at 11:00 am – 12:00 pm
Meeting format:	Face-to-face (Virtual)
RPM:	[redacted]
Preliminary Meeting Responses:	February 25, 2026

FDA Attendees:

[redacted]

Sponsor Attendees:

Background and Objectives:
Children's Hospital of Philadelphia (CHOP) submitted a meeting request on December 30, 2025, to seek FDA input on nonclinical proof-of-concept and efficacy data; the proposed definitive animal study, proposed assessment of potential off-target editing, proposed Chemistry, Manufacturing, and Controls, and proposed clinical study. The pre-meeting materials were submitted on January 28, 2026.

FDA provided its preliminary meeting responses to Children's Hospital of Philadelphia (CHOP)'s questions on February 25, 2026. After reviewing the preliminary meeting responses, CHOP notified FDA on February 26, 2026, of its decision to limit the meeting to discuss only Introductory Comments, and Questions 7, 9, 1, 2, 4 and 6.

Sponsor Questions:

Introductory Comments:
As each urea cycle disorder (UCD) is a distinct genetic disease with differences in clinical symptoms or spectrum of clinical symptoms among the different UCDs, there may be different safety and/or efficacy considerations specific to each product/disease pair. Therefore, a separate IND and BLA should be filed for each gene-specific product targeting a given UCD. However, you may submit a single master clinical protocol to evaluate the safety and efficacy of different gene-specific products across the various UCDs. As a new IND is initiated for additional gene-specific products for new UCDs, the master clinical protocol can be amended accordingly and cross-referenced. We note that the proposed approach to provide data on 5 patients with at least 3 different UCDs would not be sufficient to support a single BLA submission for all CHOP.UCD.PE products for a broad indication of all 7 UCDs, and it is expected, at this time, that a separate BLA may be required for each specific UCD. You may be able to leverage some of the non-clinical, CMC, or clinical information submitted in the first IND (or later BLA) to support subsequent submissions, as applicable.

To ensure clear communication during this meeting and in your future INDs, we will be using the following terminology to refer to the set of products that you propose:

- What you refer to as AAV.UCD.PE is a set of seven related but independent AAV drug products, with each drug product targeted to edit a specific UCD gene. Within each of these independent AAV drug products that target a specific gene, there may be multiple drug product variants that target specific mutations within that gene by encoding mutation-specific pegRNAs and ngRNAs. We recommend that you give these seven AAV drug products distinguishing names.

- What you refer to as LNP.UCD.PE is a single drug product that will be used across all seven INDs. This LNP drug product will have at least three variants that encode nickases with differing PAM preference.

Thus, your full proposed set of seven INDs will include seven independent gene-specific AAV vector drug products, each with multiple individualized mutation-specific variants, and one LNP drug product that has multiple nickase variants.

Meeting Discussion for Introductory Comments:
FDA agreed that the proposed single-arm trial under a master protocol can serve as the single adequate and well-controlled investigation to support a future BLA. FDA expects that each IND/BLA submission should include clinical data for the specific urea cycle disorder (UCD) for which the Sponsor seeks approval. FDA will be flexible in the minimum number of patients enrolled within the master protocol which will depend on the results observed within each UCD and across all UCDs (e.g., magnitude of treatment effect, safety risks etc.) and the rarity and availability of patients within each UCD. Ultimately, the totality of evidence for both efficacy and safety obtained from all

UCD patients in the master protocol will be considered and FDA will provide regulatory flexibilities when possible. FDA encouraged continued communication during the clinical development process, including the Sponsor's plan to request an end-of-phase 2 meeting,

Sponsor Question 1: *Does the Agency agree that cellular studies, rather than humanized mouse studies, will provide sufficient proof-of-concept (POC) data to support the administration of each variant of the CHOP.UCD.PE treatment to infantile-onset UCD patients?*

FDA Preliminary Meeting Response to Sponsor Question 1:
We cannot yet agree that the in vitro and in vivo POC studies described for the CHOP.UCD.PE DP targeting the ASS1 G390R mutation (pages 32-36) are sufficient to support the activity of CHOP.UCD.PE product in your initial IND submission. While we agree that the biological activity of each subsequent DP can be established via in vitro cellular studies, additional studies are needed to support the activity of CHOP.UCD.PE DP for your proposed first-in-human (FIH) clinical trial. We are concerned that you have not provided data correlating the percent editing to durable target protein expression in a sufficient number of animals dosed with the CHOP.UCD PE DP. Therefore, you should conduct a comprehensive POC study in an animal model that is amenable to on-target editing (i.e., heterozygous G390R humanized mice). We have the following comments regarding the design of this additional POC study:

1. We note that each data point in Figure 12 (page 34) appears to represent a technical replicate from the same animal. Please include adequate numbers of animals of both sexes per group in your pivotal POC study to allow for robust analysis of the data.

2. Please include CHOP.UCD.PE DP dose levels that bracket the proposed clinical dose level range and identify the minimum effective dose level in heterozygous G390R humanized mice, which appears to be 1E11 vg/kg for the AAV.UCD.PE DP and 0.5 mg/kg for the LNP.UCD.PE DP (Figure 12B, page 34). Sufficient data (e.g., % on-target gene editing and ASS1 protein expression) should be provided to support the dose level rationale for all subsequent DP variants.

3. In addition to on-target prime editing, please perform the following assessments in the liver to support the biological activity of CHOP.UCD.PE DP:

    a. Please characterize the level of transgene expression of LNP.UCD.PE DP (i.e., prime editor protein) and AAV.UCD.PE DP (i.e., pegRNA and ngRNA).

b. Please assess ASS1 mRNA and protein expression levels in heterozygous G390R humanized mice and compare to ASS1 expression in wildtype animals.

c. To support potential redosing of the LNP.UCD.PE DP in the proposed clinical study, please include a group of animals with a repeat dosing regimen. Sufficient data should be provided to support the dose level selection and time interval between LNP.UCD.PE DP doses.

d. All POC assessments should be conducted at baseline and at multiple timepoints following CHOP.UCD.PE DP administration to capture the peak and plateau of gene editing activity. Please provide your justification for the selection of each timepoint.

4. Although you propose to assess biodistribution (BD) in wildtype nonhuman primates (NHPs), BD related to on-target editing can only be assessed in humanized mice bearing the DP-targeted mutation. Please conduct the following assessments following both single and repeat dosing of the LNP.UCD.PE DP:

   a. Please evaluate on-target intended editing, synonymous editing, nonsynonymous bystander editing, or undesired indels (insertions/deletions) in a panel of tissues where significant BD of the prime editor and pegRNA/ngRNA was observed. These analyses should also be conducted in the gonads to support the assessment of developmental and reproductive toxicity (DART) risks associated with the CHOP.UCD.PE DP.

   b. In addition to your proposed PHH genomic insertion analysis, we recommend insertional mutagenesis evaluation in all tissues where there is significant AAV/LNP BD and on-target editing.

   c. Please use an appropriately designed targeted next-generation sequencing (NGS)-based method(s) to assess the edit profile at both on-target and any detected off-target sites in mouse tissues that were assessed for the proposed time interval. Please provide a detailed description of this study and report the findings of the study as a list of edit outcomes at on-target and off-target sites and the associate frequencies at different timepoints. When reporting the findings of this study, please use the data to justify the adequacy of the duration of the study or the need for additional assessments at later timepoints.

5. If the dose levels for each subsequent DP variant can be established from in vitro data collected with that variant and in vivo data from this pivotal POC study, additional in vivo studies conducted in humanized mice are not necessary. Please refer to our response to your Question #5 regarding DP

dose level extrapolation from the nonclinical in vitro studies to the proposed clinical trial.

Meeting Discussion for Sponsor Question 1.2 and 1.3b:
The Sponsor clarified that on-target editing in heterozygous ASS1 G390R humanized mice is unlikely to change the mRNA levels as the DP will correct a missense mutation, and protein levels may not increase linearly due to background expression of the normal protein at baseline. FDA agreed that characterization of the mRNA levels is likely uninformative but continued to recommend that the Sponsor assess ASS1 protein expression to confirm downstream effects of functional editing.

Meeting Discussion for Sponsor Question 1.3d:
The Sponsor requested clarification as to why multiple timepoints were needed following CHOP.UCD.PE DP administration. FDA clarified that multiple timepoints should be selected to demonstrate peak and plateau of editing activity; however, an extensive kinetic study is not necessary. For the single-dose regimen, at least one additional timepoint should be selected. For the repeat-dose regimen, multiple timepoints may be needed to support the selection of the dosing interval for CHOP.UCD.PE DP administration.

Meeting Discussion for Sponsor Question 1.4b:
The Sponsor proposed to perform long-read sequencing at the on-target site in mouse tissues where on-target editing is detected to evaluate the risk of insertional mutagenesis. FDA agreed that the Sponsor's proposal was reasonable.

Sponsor Question 2: *Does the Agency agree that the proposed definitive toxicology study of one variant of the CHOP.UCD.PE treatment (AAV.UCD.PE and LNP.UCD.PE DPs) in wild-type nonhuman primates (NHPs) and existing biodistribution data from prior studies of AAV8 and the proposed LNP formulation will provide sufficient data to support IND applications for all variants of the CHOP.UCD.PE treatment?*

FDA Preliminary Meeting Response to Sponsor Question 2:
We cannot yet agree that the proposed definitive toxicology and biodistribution study of one variant of CHOP.UCD.PE in wild-type NHPs described on pages 38-41 of the briefing package and the Study Synopsis for Study #CHOP-UCD-PE-1 are sufficient to support IND submissions for all CHOP.UCD.PE products. We have the following comments:

1. You propose to administer AAV.UCD.PE at a dose level of 1E14 vg/kg [redacted]. You also propose to administer LNP.UCD.PE at 2.4 mg/kg in the single dose BD/toxicology study [redacted]. We are concerned that the proposed dose levels of each DP may not be well-tolerated in NHPs due to significant hepatic toxicity. We recommend that you include AAV.UCD.PE and LNP.UCD.PE dose levels that

bracket the proposed clinical dose level range to identify the NOAEL for the CHOP.UCD.PE DP.

2. We do not agree with your proposed plan to reference published AAV8 BD studies (page 44) in lieu of conducting BD studies for AAV.UCD.PE to support the IND submission for the CHOP.UCD.PE DP. In addition to the route of administration and AAV serotype, AAV vector BD may be influenced by vector titers, titering methodology, product formulation, manufacturing process, the dose level administered, and the dosing regimen. If you plan to compare BD across programs, please provide a tabulated list comparing these parameters between AAV.UCD.PE and the other AAV8 drug products. Alternatively, we recommend that you incorporate BD assessment for AAV.UCD.PE into the planned toxicology study in NHPs. We have the following comments regarding BD assessment for AAV.UCD.PE:

   a. Please evaluate the BD profile and the kinetics of transgene expression in the serum and major perfused organs at multiple time points following administration of AAV.UCD.PE. Please provide your rationale for the selection of your proposed sacrifice time points.

   b. For all samples that are positive for AAV.UCD.PE presence, AAV.UCD.PE transgene levels should be measured. If a particular tissue/biofluid is negative for AAV.UCD.PE at a specific time point, then that respective tissue/biofluid does not need to be analyzed at later time points. If a particular tissue is determined to be negative upon PCR analysis, then that respective tissue does not need to be analyzed for transgene expression. However, all tissues, whether analyzed or not, should be archived for possible future analysis.

   c. For more information on BD study design, please refer to the document titled, S12 Nonclinical Biodistribution Considerations for Gene Therapy Products: Guidance for Industry (May 2023), available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/s12-nonclinical-biodistribution-considerations-gene-therapy-products.

3. Please assess the persistence of the prime editing protein in all tissues where high levels of LNP.UCD.PE BD were detected.

4. Please provide data evaluating the degradation profile of all novel LNP components in appropriate biosamples (e.g., liver S9 fractions, microsomes, hepatocytes) from mice, NHPs, and humans to assist in translation of metabolism and clearance data from the selected animal models to humans.

5. You state that germline transmission testing of CHOP.UCD.PE is not necessary because editing would require both the presence of the PE6c

mRNA from the LNP.UCD.PE DP and pegRNA/ngRNA expression from the AAV.UCD.PE DP in the same germ cells, and that prior studies indicate that AAV8 does not distribute into the germline (page 45). We do not yet agree with this conclusion due to the following gaps in the pre-IND meeting package: a) BD of both LNP.UCD.PE and AAV.UCD.PE to the gonads has not been adequately evaluated, and b) potential DART risks attributed to the LNP formulation, irrespective of editing, are unclear. We recommend a tiered approach to address the need for the conduct of DART studies. For example, data obtained from the AAV.UCD.PE BD study discussed in our Comment #2 above, the on-target editing assessment in the gonads of G390R humanized mice discussed in our Comment #4a to your Question #1, and your proposed definitive BD study of LNP.UCD.PE will help to inform these decisions. In your IND submission, please provide a comprehensive discussion, including summaries of supporting data, to facilitate further interaction on this issue.

6. Please include evaluation of complement activation and innate immune response at multiple time points within the first week post-dosing of each DP (i.e., AAV.UCD.PE and LNP.UCD.PE). Please provide your rationale for the time points and parameters selected.

7. We note that the proposed clinical immunosuppression (IS) regimen [redacted] [redacted], which are not included in the IS regimen in your pivotal toxicology study in NHPs. Please provide your justification for the IS regimen in your proposed toxicology study in NHPs and a discussion of how the selected regimen informs your proposed clinical trial.

Meeting Discussion for Sponsor Question 2.1:
FDA agreed that the Sponsor's proposed adaptive study design to identify the NOAEL was reasonable.

Meeting Discussion for Sponsor Question 2.2a and 2.3:
FDA agreed that the Sponsor's proposal to assess AAV8 vector biodistribution, AAV8 transgene expression, and prime editor protein expression at the scheduled termination timepoints in the proposed NHP study to be reasonable. FDA clarified that if these biodistribution studies do not capture the peak and plateau of transgene expression, additional studies at longer time points may be warranted, which may be conducted in heterozygous G390R humanized mice.

Sponsor Question 3: *Does the Agency agree that the proposed definitive toxicology study of one variant of the CHOP.UCD.PE treatment in wild-type NHPs will provide sufficient data to support re-dosing of patients with the LNP.UCD.PE DP component?*

FDA Preliminary Meeting Response to Sponsor Question 3:
Yes, we agree that the proposed definitive toxicology study of one variant of the CHOP.UCD.PE DP in wild-type NHPs supports re-dosing of subjects with the LNP.UCD.PE DP. In your IND submission, please provide your justification for the

selection of the time interval between doses. We also refer you to our Comment #3 to your Question #1.

Meeting Discussion for Sponsor Question 3:
There was no discussion of this question during the meeting.

Sponsor Question 4: *Does the Agency agree that the proposed off-target editing studies of a given variant of the CHOP.UCD.PE treatment will provide sufficient data to support the administration of that variant of the CHOP.UCD.PE treatment to infantile-onset UCD patients?*

FDA Preliminary Meeting Response to Sponsor Question 4:
Your proposed methods for off-target editing assessment are generally acceptable but we need additional information to assess the adequacy of your proposed studies. Specifically, we are concerned that DNA nicking at target site(s) may potentially result in an unintended adeno associated viral (AAV) vector integration event. Additionally, it is not clear what concentrations of the editor components will be used in the confirmatory studies. Please address the following in your IND submission.

1. Please clarify the reverse transcriptase template length in the final DP that you plan to use for each mutation that you intend to correct.

2. Regarding your rhAmpSeq studies, please address the following:

   a. You proposed to perform rhAmpSeq using EC90 concentrations of editor components to assess the editing rates at all the nominated off-target sites in engineered HuH-7 cells. However, it is not clear what specific dose/concentrations of AAV and LNP you plan to use for this study and why these concentrations were selected. Please provide information on target site editing activity for the proposed dose/concentrations of AAV and LNP and provide justification to support the adequacy of rhAmpSeq study.

   b. Additionally, we recommend that you use a strategy that accounts for multiple LNP dosing strategy. Please submit a detailed report of rhAmpSeq study that includes information on the number of sample replicates used, concentration of editor and related components used, on-target editing rates achieved, off-target editing rate measured, and read counts at respective on and off-target sites.

3. Please clarify if you plan to prioritize or subset nominated off-target sites for confirmatory testing study using rhAmpSeq. Please provide details of your off target edit site prioritization strategy with justification to support the adequacy of your approach.

4. Regarding your on-target edit site assessment strategy, it is not clear if your approach allows for measurement of editing activity at both the target site and native genomic site. Additionally, we are concerned about a potential AAV integration event at target site where DNA nicking event is intended. Please address the following:

    a. Please clarify if your proposed engineered HuH-7 cells harbor a sequence cassette with mutation(s) and harbor the native UCD gene sequence(s). Please use multiple sample replicates and an on-target edit site analysis strategy such as designing appropriate primers to enable independent measurements of edit outcomes at the two distinct genomic locations. Additionally, when reporting the on-target site edit rates, please provide a breakdown of editing rate resulting in mutation correction, synonymous nucleotide changes, and any other unintended nucleotide changes at the target site (i.e., mutation harboring sequence) and native genomic site.

    b. Please use an appropriate long-read sequencing-based method to assess AAV integration event(s) at target site(s) post-prime editing. Please provide a detailed report describing this study that includes information on the length of the amplicon sequenced, sequencing quality/depth acceptance criteria applied, total reads passing the acceptance criteria, and read counts supporting AAV integration event at the target site (i.e., mutation harboring sequence) and native genomic site.

    c. Please report all the observed edit outcomes (including AAV integration events) and associated read counts in a tabulated CSV or Microsoft Excel file format.

5. Please provide an annotated list of all the off-target edit sites that you identified from each off-target editing assessment method with your off-target study reports. The annotation information should clarify whether an off-target site is intergenic, intronic, exonic, or impacts splicing. For exonic sites, please indicate the impact on the amino acid sequence. Please submit this information in a tabulated CSV or Microsoft Excel file format.

6. Regarding your AAV integration site analysis (ISA), you stated that you will be using a saturating dose of AAV. It is not clear what a saturating dose of AAV means. Additionally, it is also not clear why a saturating dose will be used in this study. Please provide justification with data to support your strategy. Finally, it is not clear if the cells transduced with AAV at saturating dose will be edited using the LNP- prime editor. We recommend that ISA analysis be performed with multiple replicates using relevant sample types harboring the target sequence. The relevant sample types should be transduced with AAV followed by transfection with LNP to express prime editor to enable DNA nicking event(s). Please provide a detailed report of ISA and report an annotated list of all the AAV integration sites in the genome, at target sites.

Additionally, please report the associated read counts supporting each AAV integration event. Please submit this information in a tabulated CSV or Microsoft Excel file format.

Meeting Discussion for Sponsor Question 4.2a and 4.6:
The Sponsor clarified that the EC90 concentration will be determined for editor components for each drug product. The Sponsor also clarified that their proposed saturating concentration of editor refers to EC90 concentration. FDA stated that the Sponsor proposal to use EC90 concentration of for rhAmpSeq and ISA is acceptable. FDA recommended that Sponsor use the data and results acquired from their ISA to support the adequacy of their study.

Sponsor Question 5: *Does the Agency agree that the overall nonclinical development plan is sufficient to support IND applications for all variants of the CHOP.UCD.PE treatment?*

FDA Preliminary Meeting Response to Sponsor Question 5:
We cannot yet agree that the nonclinical data provided to date in your pre-IND meeting package are sufficient to support administration of all drug products and/or variants in a FIH clinical trial. For each DP and/or variant, please provide the following information in your IND submission:

1. Dose level extrapolation from in vitro data demonstrating product activity (e.g. % editing of HuH-7 cells transduced with a lentiviral vector carrying the target mutation following incubation with the DP) to safe and potentially biologically active dose levels proposed for the FIH clinical trial. Please justify your method of dose extrapolation and provide a sample calculation, as applicable.

3. We recommend that you conduct the following studies to evaluate the potential genotoxicity of LNP.UCD.PE DP: a) an in vitro micronucleus test and b) an in vitro mammalian cell hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene mutation test or a mouse lymphoma assay. If any positive signals for genotoxicity are detected, we recommend that you also conduct an in vivo micronucleus test. The following document may be referenced for

additional guidance on this assay: Guidance for Industry S2(R1) Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use (https://www.fda.gov/media/71980/download).

Meeting Discussion for Sponsor Question 5:
There was no discussion of this question during the meeting.

Sponsor Question 6: *Does the Agency agree that the proposed quality of materials and specifications for release testing of each mRNA DS, LNP.UCD.PE DP, and AAV.UCD.PE DP are acceptable to support IND applications for the CHOP.UCD.PE treatment?*

FDA Preliminary Meeting Response to Sponsor Question 6:
We generally agree that the proposed quality of materials and specifications are acceptable to support the submission of the IND for the initial CHOP.UCD.PE product. However, we note you plan to request an end of Phase 2 (EOP2) meeting after the completion of the proposed Phase 1/2 study to discuss the possibility of using the Phase 1/2 data to support accelerated approval for a BLA. Please be advised that in this case the CMC information for the Phase 1/2 study should be appropriate for late-stage clinical development.

We acknowledge that you propose to submit the CMC information for mRNA drug substances in a Type II master file (MF), and we acknowledge the letter of authorization (LOA) cross-referencing [redacted] for CMC information for the AAV.UCD.PE drug products (DP). Please note that the CMC readiness of these master files to support the proposed clinical development plan will be communicated directly to the MF holder via a separate communication.

Based on the information provided in the meeting package, we have the following comments for you to consider in your IND submission. While not all of these comments are absolutely necessary for an early phase clinical trial to establish safety of the product, we highly recommend that you implement our advice to support your clinical development program for accelerated approval.

1. Regarding the vector genome titer assay for AAV.UCD.PE, based on the information in the meeting package, we are unable to determine whether dosing will be based on the measured strength (i.e., vg/mL concentration) of each individual DP lot or on a target (nominal) strength. You should design your DP manufacturing process to achieve a nominal target concentration and use nominal titer-based dosing in your clinical study. The DP vector genome concentration AC should have a narrow range (e.g., nominal concentration ± 15%) to limit variability in dosing among subjects who receive different lots of DP. The experience gained during the clinical study with dosing using a nominal concentration will be needed to support commercial product labeling.

2. To ensure an accurate dose of each DP is administered in a clinical study, the dose determining assay must be qualified and must be shown to have adequate performance prior to conducting early phase clinical studies. However, if you intend to use the Phase 1/2 clinical data to support accelerated approval, the assay to determine strength should be validated prior to the initiation of the clinical study that is intended to provide the primary evidence of effectiveness to support a marketing application. Please perform a qualification or validation study to demonstrate that the assay has adequate performance, as applicable.

    a. The study should be performed using a product-specific test material.

    b. Please provide a detailed protocol for the study and the SOP for the assay.

    c. Please provide the full study report with data documenting accuracy, precision (repeatability and intermediate precision), specificity, range, linearity, limit of detection, and robustness, as applicable. The coefficient of variation (CV) for intermediate precision should be ≤ 15%. A precise assay is necessary to ensure that subjects receive the intended doses, to support consistent dosing throughout the clinical study, and to monitor product stability.

    d. Please describe any deviations that occurred during the study.

    e. To ensure consistent dosing between clinical and preclinical studies, we recommend using the same assay for measuring the DP concentration of the preclinical and clinical lots.

4. We note several acceptance criteria in the lot release specifications and stability testing protocols are not specified, including, “report results”, “client specified”, and “TBS”. To support the proposed clinical development plan, we recommend that you leverage experience from the manufacture of preclinical, developmental, or engineering lots as well as experience from other related products to set lot release acceptance criteria. The initial lot release acceptance criteria may be set relatively wide based on your limited process development and manufacturing experience and should be tightened as you gain additional product manufacturing experience.

5. We note you provide the lot release specification for the linearized plasmid DNA template in Table 10 of the meeting package; however, this table does not include a test for bioburden. We recommend that you include a test for this safety related quality attribute in the linearized plasmid DNA template lot release specification.

7. Regarding the LNP.UCD.PE DP lot release specification, please consider the following comments:

   a. We note you have not indicated if you are conducting Limulus amebocyte lysate (LAL) kinetic chromogenic method for endotoxin testing per USP <85>. Please clarify if this method adheres to USP, if not, please provide data demonstrating method sensitivity with a sample containing your matrix.

   b. We note you provide a description of the potency assay based on lentivirus transduced HuH-7 cell line for the assessment of the potency of the LNP.UCD.PE DP. However, this test is not included in the LNP.UCD.PE DP lot release specification. Please include this test in the LNP.UCD.PE DP lot release specification provided in Table 15.

8. Regarding the AAV.UCD.PE DP lot release specification, please consider the following comments:

   a. We note you do not propose testing for subvisible particulates. Testing for subvisible particulates is recommended for injectable products according to USP. We recommend that you test the DP for subvisible particulates as part of lot release and designate an appropriate acceptance criterion.

   b. We note you do not propose testing for capsid identity. We recommend that you include a capsid identity test in the AAV.UCD.PE DP lot release specification that is capsid-specific to ensure the correct serotype and prevent potential mix-ups with other AAV capsids that may be manufactured at the same facility.

   c. We note you have not indicated if you are conducting Limulus amebocyte lysate (LAL) kinetic chromogenic method for endotoxin testing per USP <85>. Please clarify if this method adheres to USP, if not, please provide data demonstrating method sensitivity with a sample containing your matrix.

   d. We note you do not propose testing for AAV aggregation. Since AAV aggregation may impact product quality and has the potential to increase immunogenicity, we recommend that you include a measure of AAV aggregation in the DP lot release specification and stability testing protocols.

   e. We note you provide a description of the potency assay based on lentivirus transduced HuH-7 cell line for the assessment of the potency of the AAV.UCD.PE DP. However, this test is not included in the AAV.UCD.PE DP lot release specification. Please include this test in the AAV.UCD.PE DP lot release specification provided in Table 17.

   f. Based on the information provided in the meeting package, it is unclear if poloxamer-188 (P188) is used in the DP manufacturing process. If the formulation buffer contains P188, its concentration in the DP should be controlled to ensure adequate quality and stability of your product. Please develop an assay and acceptance criterion for poloxamer-188 concentration, if applicable.

9. Regarding the long-term stability testing plan for AAV.UCD.PE DP, please consider the following comments:

   a. We note that you are using sterility testing as part of your stability testing program for the AAV.UCD.PE DP. Although sterility is an important attribute for stability, sterility testing itself has limited value as part of a stability program, we recommend using container closure integrity testing

(CCIT) annually and at the final time point. For more information, please refer to "Container and Closure System Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products", dated February 2008 (https://www.fda.gov/media/76338/download).

b. We note you do not propose testing for AAV aggregation. We recommend that you include a test for AAV aggregation with a quantitative acceptance criterion in the long-term stability testing plan.

12. We note the AAV.UCD.PE plasmid sequence includes a stuffer sequence . Please provide the complete stuffer sequence with analysis identifying any open reading frames (ORFs). If any ORFs are identified, please provide a safety assessment addressing potential expression and associated risks.

Meeting Discussion for Sponsor Question 6.2:
The Sponsor asked whether retrospective validation of the strength determining assay using the Phase 1/2 DPs would be acceptable, and whether the same considerations apply to the validation of other assays to support a BLA. The FDA clarified that retrospective validation is not acceptable. If Phase 1/2 data will be used to support efficacy for accelerated approval, the strength determining assay must be validated prior to initiation of the study to ensure adequate performance and confidence in the administered dose. All other assays must be qualified prior to initiation of the study and validated prior to initiating the PPQ protocol. The Sponsor acknowledged.

Meeting Discussion for Sponsor Question 6.8f:
The Sponsor asked whether a poloxamer-188 (P188) concentration assay is required for all AAV DPs in the Phase 1/2 study. The FDA clarified that performing the P188 concentration assay as a characterization test may be acceptable for the Phase 1/2 study. The FDA recommended the Sponsor collect P188 concentration data during clinical development to demonstrate adequate process control and establish a P188 release specification for the BLA. The Sponsor acknowledged.

Sponsor Question 7: *Does the Agency agree that the overall chemistry, manufacturing, and controls plan could be sufficient to support an accelerated approval of the CHOP.UCD.PE treatment?*

FDA Preliminary Meeting Response to Sponsor Question 7:
We acknowledge the challenges presented by the rare nature of UCD and the individualized nature of the CHOP.UCD.PE products. However, based on the information provided in the meeting package, we cannot agree at this time that the overall CMC plan is sufficient to support a BLA submission (including accelerated approval) for the CHOP.UCD.PE products. Please be aware that manufacturing in a BLA submission must comply with CGMP, as required by section 501(a)(2)(B) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 351(a)(2)(B)). CMC development should evolve concurrently with clinical development. Given the shortened clinical investigation phase expected for individualized therapies for rare diseases, we have the following comments for you to consider in preparation of your IND submission and during clinical development (Please see also FDA response to Question 6).

1. We note you propose to use data from the production of multiple individualized CHOP.UCD.PE products and/or variants, manufactured for subjects in the clinical study, to support process validation rather than manufacturing multiple batches of the same variant solely for validation purposes. We agree that it is not necessary to manufacture identical variant DP lots for process performance qualification (PPQ). It may be more appropriate to use data from different DP variants to support process validation and product release specifications, provided you demonstrate that the manufacturing process is consistent across products and/or variants and that differences in AAV vectors and mRNA sequences do not impact process performance or product quality.

2. To align your CMC development with your clinical development timeline goals, we recommend that you define the commercial manufacturing process early in development and use knowledge gained during nonclinical studies and manufacturing experience from engineering lots and early DP lots in the proposed Phase 1/2 study, to identify critical quality attributes (CQAs) and critical process parameters (CPPs). You may then use some of the later DP

lots manufactured for subjects in the Phase I/II study as PPQ batches to demonstrate manufacturing process consistency and process validation.

a. PPQ studies should be designed based on a risk assessment, and PPQ for individualized products requires careful consideration of the potential effects of the variable features on process performance. We recommend that the process validation protocol clearly define all product attributes and process parameters to be evaluated, acceptance criteria and parameter ranges, and the number of PPQ batches to be manufactured. The specific number of PPQ batches should be justified based on your overall process understanding, manufacturing experience, and risk assessment. We recommend you submit this protocol to the FDA for review and feedback as early as feasible during product development.

b. Please be advised that DP lots manufactured for subjects in the Phase 1/2 study as PPQ batches should be manufactured with enhanced in-process sampling and testing to support process validation and qualification. To support the validation campaign, appropriate in-process acceptance criteria and controls must be established, and in-process stability studies must be performed to support manufacturing hold times of process intermediates and the DS. Please also describe the individual in-process hold time ranges used in manufacture, your approach to evaluating the upper limits of these ranges, and the cumulative manufacture time allowed.

c. We recommend that you consult the review team when planning process validation and PPQ studies.

3. Please be aware, for a BLA submission all analytical methods used to support product quality and process control must be fully validated. All analytical method validation protocols and final study reports should be included in your BLA. The method validation descriptions/protocols/reports should include sampling procedures, all appropriate performance parameters, and information on assay suitability and controls.

4. Based on the information provided in the meeting package, it is unclear if the CHOP CVC facility will function as the commercial manufacturing facility for the CHOP.UCD.PE products. In general, the production of an investigational drug product for use in a Phase 1 clinical trial is exempt from compliance with the regulations in 21 CFR Part 211. This exemption generally applies to studies that are designed to establish basic safety, rather than efficacy of the drug product. Since you propose to use the data from the Phase 1/2 study to support a license application, this exemption from compliance with Part 211 may not apply. If you intend to use DP lots manufactured for subjects in the Phase 1/2 study as PPQ batches to support a future BLA, the CHOP CVC

facility must be fully CGMP-compliant and inspection-ready prior to manufacturing these lots.

5. Please be advised that changes to your manufacturing process or facility could potentially impact product quality and will require demonstration of product comparability. We recommend that you establish the commercial manufacturing process and facility early in development to minimize the need for extensive comparability studies prior to BLA submission. We strongly recommend that optimization of the process or changes to the process should be minimized once the pivotal trials are initiated.

6. We note you plan to perform stability testing for the first produced version of the AAV.UCD.PE DP, and do not plan to conduct stability testing on other batches of DP variants. While stability data from a representative batch will be sufficient for initiation of an IND, please be advised that you will need to provide adequate stability data in your BLA to support the product shelf life. Due to the limited number of DP batches expected to be manufactured, we recommend that you develop a strategy for establishing a shelf life for the product early in development. This should include collecting stability data for all batches manufactured using appropriately validated and stability-indicating assays and investigating opportunities to leverage related product data to support and justify the proposed shelf life.

7. Please be aware that while you may cross-reference Type II master files for CMC information for the mRNA DS and AAV.UCD.PE DP in the IND submission, you will not be permitted to cross-reference these Type II master files for a future BLA submission. A license holder is responsible for controlling the entire manufacturing process for the DS and DP and all relevant CMC information must be provided in the BLA submission.

Meeting Discussion for Sponsor Question 7.4:
The Sponsor asked whether Phase 3 extension DP lots could serve as PPQ batches and whether PPQ data from one AAV.UCD.PE drug product variant could support the BLA for another UCD gene. The FDA clarified that the manufacturing process must be validated prior to BLA submission, and that PPQ batches may be manufactured across both Phase 1/2 and Phase 3 studies, provided that adequate control of the manufacturing process is demonstrated. The FDA advised that products distributed commercially must be manufactured by the commercial manufacturing process. The FDA further clarified that PPQ data from one drug product variant may be leveraged to support BLAs for other UCD genes, provided the products are manufactured using the same process at the same facility and a comprehensive justification is provided addressing similarities and differences between different drug products (e.g., the impact of sequence variation on critical quality attributes). The FDA recommended the Sponsor submit the PPQ protocol to the IND to seek further advice on their PPQ strategy, when appropriate. The Sponsor acknowledged.

Sponsor Question 8: *Does the Agency agree that the proposed potency assays for the CHOP.UCD.PE treatment are acceptable to support IND applications for all variants of the CHOP.UCD.PE treatment?*

FDA Preliminary Meeting Response to Sponsor Question 8:
We note that your proposed potency assay for the AAV.UCD.PE DP uses a customized lentivirus-transduced HuH-7 cell line that will be generated for each subject's specific mutation(s). Based on the information provided in the meeting package, we generally agree the proposed potency assay for the CHOP.UCD.PE products is acceptable to support the initiation of IND applications for all the CHOP.UCD.PE products. However, we still have many questions for how this assay will support commercialization and have the following comments for you to consider in the IND submission and during clinical development.

1. Please provide a justification for your selection of the reference controls for the DS activity and DP potency assays, including a discussion on how the selected reference controls are appropriate for assessing the activity of all products and variants.

2. We note that for compound heterozygous patients, the AAV.UCD.PE DP will express two different pegRNAs and two different ngRNAs to target two pathogenic mutations. Please describe how potency will be measured for a DP variant targeting two pathogenic mutations and how the acceptance criterion will be applied. Your discussion should address potential differences in editing efficiency between the two target sites and potential competition or interference between the two pegRNA/ngRNA pairs for the prime editor.

3. Please describe your strategy for validating the potency assays across different products and variants that may provide prime editing solutions for both homozygous patients and compound heterozygous patients. This should include a discussion of how the assay design and acceptance criterion will accommodate these different configurations.

4. We note you propose to use the data from the Phase 1/2 study to support a license application. To support this clinical development plan, you will need to qualify and implement the potency assays at the GMP compliant QC lab prior to the initiation of the proposed Phase 1/2 study.

5. Please describe your strategy for ensuring consistent lentiviral integration copy number across different cell line constructs generated for different mutations. Variability in copy number could affect the potency assay performance across different DP variants.

6. We recommend that you consider identifying the most commonly occurring UCD gene mutations based on published literature and consider how these

could be incorporated into a single cell line that might enhance control of the potency assay design for both homozygous and compound heterozygous configurations across multiple drug products and/or variants.

7. Please describe your strategy for adding new mutations to the potency assay after the assay has been validated. This may include a discussion of whether generation of a new lentivirus transduced HuH-7 cell line will require re-validation of the assay or whether a suitability assessment will be sufficient.

Meeting Discussion for Sponsor Question 8:
There was no discussion of this question during the meeting.

Sponsor Question 9: *Does the Agency agree that the general design, including the proposed safety and exploratory efficacy outcome measures, enrollment criteria, data capture and monitoring plans, and long-term follow-up plan are appropriate for the umbrella trial protocol outlined in the protocol synopsis?*

FDA Preliminary Meeting Response to Sponsor Question 9:
We agree in principle with the proposed master protocol for an open-label, single arm study of intravenous CHOP.UCD.PE in patients with UCDs due to genetic variants amenable to corrective editing. However, you need to specify the comparator that will be used for all comparative analyses for efficacy and safety including a scientific rationale for the choice of the control group and the protocol for the collection of data in the control group. More comments may be provided with submission of a full clinical protocol. It is premature to provide detailed advice until all CMC, PT, and bioinformatics (BI) concerns are addressed, and a full clinical protocol is submitted for review.

Given the rarity of each UCD and the limited number of patients with each UCD expected to be evaluated in the master protocol, we anticipate that data from your master protocol will provide evidence to support substantial evidence of effectiveness in a future BLA. Therefore, the proposed master protocol should be designed to be an adequate and well-controlled study in accordance with 21 CFR 314.126. The use of an appropriate external control may be reasonable if a control population comparable to the treated population is identified (see comment on choice of control group). Please also refer to the FDA draft guidance for study design considerations under the plausible mechanism framework for individualized therapies, available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/considerations-use-plausible-mechanism-framework-develop-individualized-therapies-target-specific.

We agree with an End of Phase 2 (EOP2) meeting to assess clinical and CMC readiness for a potential BLA submission including the most appropriate regulatory approval pathway.

Meeting Discussion for Sponsor Question 9:
FDA clarified that for rare disease trials, natural history data and a patient's baseline state prior to receiving treatment (as proposed in the Sponsor's trial) could serve as a control, particularly when the natural history of disease is well understood.

In the IND submission, the Sponsor should provide detailed justification for the chosen comparator which may be supported by literature and disease-specific evidence. FDA recommends following guidance on optimizing diet in clinical trial (available at: http://www.fda.gov/regulatory-information/search-fda-guidance-documents/inborn-errors-metabolism-use-dietary-management-considerations-optimizing-and-standardizing-diet). Ultimately, approval decisions will be based on the totality of evidence, magnitude of treatment effect, and safety data across all patients in the master protocol.

Sponsor Question 10: *Does the Agency agree that if there is a uniform response across subjects, the proposed umbrella trial in a limited number of subjects could be sufficient for an accelerated approval of the CHOP.UCD.PE treatment?*

FDA Preliminary Meeting Response to Sponsor Question 10:
It is premature to comment on the regulatory approval pathway until alignment is reached on the trial design and other aspects of your development program. Discussion and alignment on an adequate safety database will also be needed once safety data becomes available. See FDA Response to Question 9 and Introductory Comments.

Meeting Discussion for Sponsor Question 10:
There was no discussion of this question during the meeting.